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Large-scale DNA-based typing of HLA-A and HLA-B at low resolution is highly accurate specific and reliable

Key words:
HLA-A; HLA-B; polymerase chain reaction; sequence-specific oligonucleotide typing; sequence-specific primer typing; bone marrow registry

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Abstract: DNA-based typing of HLA class I alleles of the HLA-A and HLA-B loci using sequence-specific oligonucleotide primers and/or probes has been used for the large-scale typing of individuals for the National Marrow Donor Program® unrelated donor registry. Typing was performed by 16 laboratories at a low level of resolution (e.g. A*01, B*07). The results of blinded quality control analysis for the first 12 months of the project show the typing to be highly accurate, specific and reliable. The total error rate based on 11,545 HLA-A and 11,428 HLA-B assignments was 1.1% for HLA-A and 1.9% for HLA-B. This level of accuracy is particularly remarkable because the quality control samples could not be distinguished from 64,180 donor samples tested at the same time by the laboratories.

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DNA-based typing of HLA class I alleles is a powerful and clinically important tool for identifying the extensive polymorphism of these molecules. Many approaches to DNA-based typing have been developed based upon the amplification power of the polymerase chain reaction (PCR) and the ability of sequence-specific oligonucleotide primers and probes to discriminate amongst DNA sequences differing by as little as a single nucleotide. Two commonly employed methods, amplification by sequence-specific oligonucleotide primers (SSP) and hybridization of sequence-specific oligonucleotide probes (SSOP), have been applied to large scale typing of donors for the National Marrow Donor Program® unrelated donor registry. The SSOP approach was previously applied to HLA-DR and -DQ loci and the quality control analysis of these typing results has been previously reported (1, 2). We report here the results of our quality control analysis for the first 12 months of the project in which Class I typing results from 64,180 donors were obtained.

Mat rial and m th ds

Quality control cells

Whole blood was collected from newly recruited donors in acid citrate dextrose (ACD) using standard phlebotomy procedures. Aliquots (1 ml) of whole blood were stored frozen at −20°C or −60°C and were shipped to each laboratory for HLA typing. Each laboratory received 50–200 donor samples each week with typing results due within 14 days of sample receipt. In addition, approximately 10% of the samples in each weekly shipment were quality control whole blood samples which could not be distinguished from the donor samples. The quality control samples were initially typed for HLA-A,-B by SSOP and/or sequence-based typing in a reference laboratory which was not involved in the registry typing and the typings were confirmed by consensus with the participating laboratory assignments.

The 141 whole blood donors of the quality control samples were selected based on availability without regard for HLA types. Samples were sent randomly to the laboratories with a policy that no laboratory would receive the same quality control sample within any three month time period. Because the quality control panel was assembled over the year, quality control cells were typed from 1 (1 cell) to 67 times resulting in assignments for a total of 5,803 samples. Forty-nine cells were typed by all 16 laboratories; however, most of the quality control samples were not typed by all of the laboratories during the first 12 months. Table 1 shows the distribution of HLA-A and HLA-B types in the quality control typing. The panel included 17 of the 21 HLA-A types and 29 of the 36 HLA-B types. The cells included 5 of the 21 possible HLA-A homozygous types (e.g. A*02 only) and 6 of the 36 possible HLA-B homozygous types. The panel also included 49 of the 210 different HLA-A heterozygous combinations (e.g. A*02, A*24) and 81 of the 630 HLA-B heterozygous combinations.

Refer nce cell panel

DNA from a reference B-lymphoblastoid cell panel was used as an internal quality control to give both positive and negative results with most primers and probes for SSP and SSOP testing. Each membrane tested by SSOP included the entire panel of reference DNA for that set of HLA-A or HLA-B probes. A set of 12 samples carrying various HLA alleles was used in each assay to monitor the accuracy of one of the two SSP methods, the exonuclease-released fluorescence method. The accuracy of the second SSP method was monitored by quality control with reference cell lines each time a new lot of SSP trays were aliquoted.

HLA-A and -B assignments and frequency [a] in the quality control panel

HLA-A type	No. times typed (Frequency in %)	HLA-B type	No. times typed (Frequency in %)
A*01	1514 (13.0)	B*07	1392 (12.0)
A*02	2977 (25.7)	B*08	1098 (9.5)
A*03	1393 (12.0)	B*13	509 (4.4)
A*11	784 (6.8)	B*14[b]	337 (2.9)
A*23	652 (5.6)	B*15 group 1[c]	1064 (9.2)
A*24	1260 (10.9)	B*15 group 2[c]	168 (1.4)
A*25	123 (1.1)	B*18	453 (3.9)
A*26	245 (2.1)	B*27	518 (4.5)
A*29	220 (1.9)	B*35	1042 (9.0)
A*30	373 (3.2)	B*37	37 (0.3)
A*31	265 (2.3)	B*38	200 (1.7)
A*32	264 (2.3)	B*39	281 (2.4)
A*33	317 (2.7)	B*40[b]	496 (1.7)
A*34	0 (0.0)	B*41	156 (1.3)
A*36	0 (0.0)	B*42	139 (1.2)
A*43	0 (0.0)	B*44	1404 (12.1)
A*66	110 (0.9)	B*45	50 (0.4)
A*68	272 (2.3)	B*46	113 (1.0)
A*69	102 (0.9)	B*47	65 (0.6)
A*74	55 (0.5)	B*48	0 (0.0)
A*80	0 (0.0)	B*49	140 (1.2)
Blank[d]	680 (5.9)	B*50	159 (1.4)
Total	11,606	B*51	585 (5.0)
		B*52	84 (0.7)
		B*53	206 (1.8)
		B*54	0 (0.0)
		B*55	48 (0.4)
		B*56	91 (0.8)
		B*57	224 (1.9)
		B*58	189 (1.6)
		B*59	0 (0.0)
		B*67	0 (0.0)
		B*73	0 (0.0)
		B*78	0 (0.0)
		B*81	52 (0.4)
		B*82	0 (0.0)
		Blank[d]	306 (2.6)
		Total	11,606

[a] Frequency=Type +/n; the panel includes 5.803 cells.
[b] B*14 included alleles encoding B64 and B65; B*40, B60 and B61
[c] Alleles in the B*15 group were subdivided into two groups which approximately parallel the division between alleles specifying B15 (group 1) or B70 (group 2) antigens.
[d] Presumed homozygous samples were assigned a blank as the second type (i.e. A*02 was considered to be A*02,blank for this analysis). Of the 5.803 quality control samples, 5.9% were apparent HLA-A homozygotes and 2.6% were apparent HLA-B homozygotes.

Table 1

SSOP protocols

Genomic DNA was purified from whole blood using a variety of techniques, predominately the QIAamp blood kit (Qiagen, Valencia, CA, USA). Fourteen laboratories used an SSOP approach to type these samples. The primers used in the PCR protocol amplified exon 2, intron 2, and exon 3 of all alleles at the HLA-A or HLA-B loci. Eleven laboratories utilized primers provided by Lifecodes Corp. (Stamford, CT, USA); three laboratories utilized other primer sets (3–7). The amplification protocol varied from laboratory to laboratory and each DNA sample underwent from 25–35 cycles of amplification.

Aliquots of the amplified DNA were spotted on membranes and hybridized with a panel of SSOP. The SSOP were selected to detect HLA-A and HLA-B types described in Table 1. Eleven laboratories utilized SSOP provided by Lifecodes Corp.; three laboratories utilized other SSOP sets (3–5, 8–10). HLA-A and HLA-B consensus probes were used to monitor for DNA contamination and to monitor the amount of DNA loaded on a membrane. These probes contained sequences common to all HLA-A or all HLA-B alleles. SSOP were labeled using a variety of techniques including 32P, digoxigenin, and alkaline phosphatase. The labels were detected by either autoradiography or by incubation with chemiluminescent or chromogenic substrates. An SSP method with or without probes was used to resolve some ambiguous types in some laboratories.

SSP protocols

Two laboratories utilized an SSP approach. One laboratory used an exonuclease-released fluorescence detection system (11–13). The second laboratory used a modification of Bunce et al. (14) with 28 primer mixes for HLA-A and 56 for HLA-B.

Analysis of quality control data

Accuracy of typing carried out by all 16 participating laboratories at low resolution was monitored over a 12-month period using qual-

HLA-A correlation

QC Type	blank[b]	01	02	03	09	10	11	23	24	25	26	28	29	30	31	32	33	34	36	66	68	69	74	Sum
blank	677	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	680
01	4	1506	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	2	0	0	0	0	1514
02	6	3	2961	1	0	0	0	1	0	0	2	0	0	0	0	0	0	1	1	0	1	0	0	2977
03	4	0	1	1388	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1393
11	5	0	0	0	0	0	779	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	784
23	0	0	2	0	20	0	0	627	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0	652
24	0	0	0	1	0	0	0	5	1252	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1260
25	0	0	0	0	0	0	0	0	0	123	0	0	0	0	0	0	0	0	0	0	0	0	0	123
26	1	0	0	0	0	1	0	0	0	0	243	0	0	0	0	0	0	0	0	0	0	0	0	245
29	0	0	0	0	0	0	0	0	0	0	0	0	220	0	0	0	0	0	0	0	0	0	0	220
30	1	0	0	0	0	0	0	0	0	0	0	0	0	372	0	0	0	0	0	0	0	0	0	373
31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	263	0	2	0	0	0	0	0	0	265
32	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	260	0	1	1	0	0	0	0	264
33	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	313	0	0	0	1	0	0	317
66	0	0	0	0	0	21	0	0	0	0	3	0	0	0	0	0	0	0	0	86	0	0	0	110
68	1	0	0	0	0	1	0	0	0	0	0	8	0	0	0	0	1	0	0	0	261	0	0	272
69	0	0	0	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	1	92	0	102
74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	54	55
Sum	701	1509	2965	1391	21	23	779	634	1256	123	248	17	221	373	265	260	317	2	5	86	264	92	54	

n=11,606

[a] In some circumstances, the oligonucleotide reagents were not able to achieve the split level of resolution and the type was reported as a broad type (e.g. A*10 instead of A*26). This broad assignment was considered correct.

[b] Blank indicates that a second type was not assigned.

Table 2

		Lab type[a]																																					
		blank[b]	05	07	08	13	14	15 g1	15 g2	15	16	18	21	22	27	35	37	38	39	40	41	42	44	45	46	47	48	49	50	51	52	53	55	56	57	58	78	81	Sum
	blank	298	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	302
	07	2	0	1381	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	3	1390
	08	3	0	1	1082	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1092
	13	0	0	0	0	508	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	509
	14	2	0	0	0	0	334	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	337
	15 g1	2	0	1	0	0	1	1051	5	3	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1064
	15 g2	0	0	0	0	0	0	7	159	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	168
	18	1	0	0	0	1	0	0	0	0	0	450	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	453
	27	3	0	0	0	0	0	1	0	0	0	0	0	0	514	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	518
	35	6	0	0	0	0	0	0	0	0	0	0	0	0	0	999	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1007
Q	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37
C	38	0	0	0	0	0	0	0	0	0	22	0	0	0	0	0	0	176	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	199
	39	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	277	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	281
T	40	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	491	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	496
y	41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	153	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	154
p	42	1	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	129	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	139
e	44	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1397	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1404
	45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50
	46	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	109	0	0	0	0	0	0	0	0	0	0	0	0	0	113
	47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	65	0	0	0	0	0	0	0	0	0	0	0	0	65
	49	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	139	1	0	0	0	0	0	0	0	0	0	140
	50	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	147	0	0	0	0	0	0	0	0	0	159
	51	1	47	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	506	0	1	3	0	0	0	2	0	563
	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	83	0	0	0	0	0	0	0	84
	53	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	204	0	0	0	0	0	0	206
	55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	48	0	0	0	0	0	48
	56	0	0	1	0	0	0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	65	0	0	0	0	90
	57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	223	0	0	0	224
	58	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	188	0	0	189
	81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	52	52
	Sum	329	47	1385	1091	509	337	1060	164	4	22	450	11	25	516	1001	37	177	278	495	155	134	1401	52	111	65	1	140	148	508	83	208	54	65	223	189	2	56	

n=11,533[c]

[a] In some circumstances, the oligonucleotide reagents were not able to achieve the split level of resolution and the type was reported as a broad type (e.g. B*05 instead of B*51). This broad assignment was considered correct. B*15 g1, B*15 group 1.

[b] Blank indicates that a second type was not assigned.

[c] The table does not include typings in which the laboratory did not distinguish among 2 or 3 heterozygous types (Table 4); therefore, the total number of typings is less than the HLA-A typings.

Table 3

ity control samples which could not be distinguished from donor samples (i.e. blind samples). The consensus HLA-A,-B types expressed by the quality control cells were determined by the DNA-defined type assigned by a reference laboratory and verified by consensus of the participating laboratories which characterized the cell. Cells which only had one HLA-A or HLA-B type assigned were assumed to have a blank as the second typing for that locus, i.e. A*01 was treated as A*01, blank. The typing results reported by each laboratory for each cell were compared to the consensus HLA-A, -B types and the data are reported in Tables 2 and 3. In the instances in which a laboratory assigned both alleles incorrectly (6 times for HLA-A, 14 for HLA-B), the incorrect identifications were assigned, for the purpose of this analysis, based on structural similarity (i.e. alleles encoding antigens within the same serologic broad specificity or CREG). If there was no structural similarities, the alleles were matched in numerical order. For example, a sample which carried A*01,*69 was mistyped as A*68, blank. In this case, because A*68 and A*69 are related, the analysis assumed that A*69 was incorrectly typed as A*68 and the A*01 was typed as a blank. Alternatively, a sample which carried A*02,*24 was mistyped as A*03,*30. In this case, the alleles have no structural similarity so the analysis assumed that A*02 was mistyped as A*03 and A*24 as A*30 (i.e. numerical order). Failure by a laboratory to amplify both alleles at a locus occurred in 0.2% of the quality control samples examined. These failures were not included in the statistical analysis of the quality control data.

The analysis of the data utilized methods designed for validating screening tests (15). In some cases, the laboratories assigned "broad" HLA assignments (e.g. A*10 instead of A*66). These broad assignments were not included in the calculation of the statistics. Type positive (Type+) is defined as the total number of instances in which the HLA type occurred [1×the number of heterozygotes with the type]. Type negative (Type−) is defined as the total number of instances in which HLA types other than that particular type occurred. For example, in the 11,545 total HLA-A assignments made (n=11,545 excluding broad assignments), A*01 occurred 1,514 times (Type+) (Table 2). The number of Type− samples, therefore, is 11,545–1,514 or 10,031. Lab+ is defined as the total number of times the laboratories identified a particular type (e.g. 1,509 for A*01). Lab− is defined as the total number of times that a laboratory did not identify the type 11,545–1,509 or 10,036 for A*01. True-positive (TP) is defined as the number of instances which are Type+ and Lab+ (1,506 for A*01). True-negative (TN) is defined as the instances which are Type− and Lab− (10,028 for A*01). False-positive (FP) is defined as the instances which are Type− and Lab+. False-negative (FN) is defined as the instances which are Type+ and Lab−. The sensitivity for a particular type is calculated as TP/Type+ and is expressed as a percent. Specificity is defined as TN/Type− and is expressed as a percent. The positive predictive value (PPV) is the probability that a laboratory assertion that a type is present is true (TP/Lab+) and is expressed as a percent. The negative predictive value (NPV) is a measure of the proportion of laboratory negatives which lack the type (TN/ Lab−) and is expressed as a percent. Type correctly classified (TCC) is the summation of true-positive and true-negative divided by the total number of assignments (TP+TN/n) and is expressed as a percent. The frequency of a particular type is defined as Type+/n. The error rate is defined as the total number of false-positive and false-negative assignments divided by the total number of assignments and is expressed as a percent.

Results and discussion

The National Marrow Donor Program (NMDP) initiated a large volume, HLA class I (A and B loci) molecular typing project in 1997 aimed at characterizing the HLA types of volunteer donors for a bone marrow registry. A total of 64,180 frozen blood samples from donors were typed between July 1, 1997 and June 30, 1998. Initially, 16 laboratories participated (14 using SSOP and 2 using SSP). In the last 6 months, 13 laboratories continued on this project using either SSOP (12 laboratories) or SSP (1 laboratory). Typing was carried out at a low resolution based on the first two numbers of the allele name with the exception of B*15 which was divided into two groups of alleles (Table 1). This study describes the accuracy, specificity, and reliability of HLA-A and -B typing assignments of blinded quality control samples which were typed in parallel with donor samples during high volume testing. A total of 5,803 quality control samples were typed, reflecting approximately 10% of each laboratory's shipment volume. These samples were derived from a panel of 141 cells. There were 11,606 total assignments made for HLA-A and 11,606 for HLA-B.

Table 2 shows the correlation between laboratory and consensus HLA-A assignments for the quality control cells. Incorrect assignments of HLA-A were mainly limited to single sporadic errors. Exceptions included 5 A*24 alleles incorrectly assigned as the closely related assignment, A*23; 3 A*66 alleles assigned as A*26; and 3 A*02 alleles assigned as A*01. In some cases, laboratories assigned broad typings (e.g. A*10 instead of A*66). These assignments were considered correct and likely resulted from the failure of the laboratories to have sufficient probes or primers needed to achieve the required resolution. As shown in Table 2, types were assigned as blanks in 24 cases representing 0.2% of the total (n=11,606–680);

List of ambiguous typings

Table 4

HLA-B assignment	Ambiguous HLA-B type assigned by laboratory	No. occurrences (%*)
B*07,*08	B*07,*08 or B*07,*42	6 (0.1%)
B*07,*41	B*07,*41 or B*40,*42	2 (0.0%)
B*08,*51	B*08,*51 or B*08,*78	10 (0.2%)
B*08,*56	B*08,*56 or B*08,*55	1 (0.0%)
B*15 group 1,*35	B*15 group 1,*35 or B*15 group 1,*15 group 1	35 (0.6%)
B*15 group 1,*38	B*15 group 1,*38 or B*15 group 1,*39	1 (0.0%)
B*15 group 1,*51	B*15 group 1,*51 or B*15 group 1,*52 or B*15 group 1,*78	12 (0.2%)
B*40,*40	B*40,*40 or B*40,*47	2 (0.0%)
B*42,*42	B*42,*42 or B*42,*55	1 (0.0%)
B*42,*42	B*42,*42 or B*42,*08	1 (0.0%)

* n=5,803 individuals

types were assigned to blanks in only 3 out of 680 cases (0.4%, n=680). Failure to assign a type (i.e. a blank) likely represents a failure to achieve adequate amplification. Failure appeared to be sporadic. There was no significant association of a particular HLA-A type with failure to obtain amplification. In total, only 1.1% of the HLA-A assignments were incorrect.

Table 3 summarizes the correlation between the laboratory assignments of HLA-B and the consensus types of the quality control cells. Incorrect assignments of HLA-B included single sporadic errors and several more frequent incorrect assignments (e.g. B*15 group 1 vs. B*15 group 2; B*08 vs. B*42). In some cases, laboratories assigned broad typings (e.g. B*05 instead of B*51 or B*22 instead of B*56). In 71 cases, the laboratories did not have sufficient reagents to resolve typings and cells were assigned two alternative types, e.g. B*07,B*08 or B*07,B*42. These typings or ambiguities were not considered discrepant and are described in Table 4. Types were missed in 31 cases representing 0.3% of the total (n=11,533–302); types were assigned to blanks in only 4 out of 302 cases (1.3%, n=302). There was a significant association of B*46 with failure to obtain amplification (P<0.0001). Since the 4 failures to amplify B*46 were obtained by several laboratories testing a single quality control cell, it is not clear whether failure resulted from the properties of the B*46 allele, from the HLA-B allele combination (B*46,*15 group 1), or from the quality of the cells used for DNA extraction. In total, only 1.9% of the HLA-B assignments were incorrect.

Sensitivity is defined as the probability of testing positive if the type is present. As the sensitivity of the test increases, the number of individuals typing as false-negatives decreases. The value for each HLA-A type is greater than 96.6% (data not shown) suggesting that, at this level of sensitivity, few types are missed. HLA-B ranged from 92.8–100%. High sensitivity is particularly important for a bone marrow registry since incorrectly typed individuals carrying a specific HLA type are missed during a donor search for that type.

Specificity is defined as the probability of the test for a specific type being negative when the type is not present. A highly specific test will result in a lower proportion of individuals without the type who appear as false-positives. The values for HLA-A and HLA-B range from 99.7% to 100% (data not shown). Although less important than sensitivity, the high specificity value suggests that potential donors selected from the registry are likely to carry the types indicated.

Positive predictive value (PPV) measures whether or not an individual actually has a specific type if the test for that type is positive. Likewise, the negative predictive value (NPV) is the probability that an individual has a specific type if the test for that type is negative. These values are related to the sensitivity and specificity of the typing procedure but also to the prevalence of the type in the population. The PPV ranged from 88.9%–100% and the NPV ranged from 99.8–100% for both class I loci reflecting the utility of the DNA testing protocol. The values for the type correctly classified (TCC) range from 99.7–100% indicating the high level of accuracy of the class I typing using this methodology. It is also important to note that this accuracy was obtained by laboratories using different approaches (SSP, SSOP), reagents, and protocols in the typing procedure.

Approximately 25% of the errors were attributed by the participating laboratories to poor amplification of an allele and ~20% to sample switches. Clerical errors were ~5%. The remaining errors were due to incorrect interpretation of results which included weak probe reactivity or incorrect assignments. Errors were associated more with individual laboratories than with the typing methodology

used. During the 12-month period, the quantity and types of errors changed as technical problems such as amplification failure resulting in the loss of a single allele were identified and resolved and as poorly performing laboratories were dropped from the project. During the year, the average error rate decreased significantly from an average of 3.0% in the first three months to an average of 0.3% in the last three months.

Our data indicate that the SSP and the PCR/SSOP techniques utilized are highly accurate, specific and reliable. These results are further strengthened by the fact that the laboratories could not discriminate the quality control samples from the donor samples during this high volume testing. One limitation of this study, however, is the characteristics of the quality control cell panel. Because of the need to utilize blood samples which were indistinguishable from donor samples and to include cells with known DNA-based HLA-A,-B types, the current panel was selected based on availability. Inclusion of all HLA-A,-B low-resolution types and a greater diversity of heterozygous combinations would strengthen the evaluation of typing quality. A second study evaluating the accuracy of the DNA-based class I registry typing compared to the serologic typing of the same cells is currently underway. Because that study evaluates the typing of volunteer donors from U.S. minority populations, the quality of the typing of a diverse pool of HLA alleles and combinations will be determined (H. Noreen, in preparation). On the whole, these data show that it is feasible to perform high quality DNA-based HLA class I typing on a large scale. This establishes DNA-based typing as the method of choice for certain HLA typing applications.

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